

I. AMENDMENTS TO THE CLAIMS

Please cancel claims 33 to 35, without prejudice or disclaimer.

Please amend claims 21, 22, 23, 26, 30, 31, 32 and 36 by deleting the stricken material and inserting the underlined material as follows:

21. (Twice Amended) A method for detection and differentiation of pathogenic enterobacteria in a sample, said method comprising:

isolating nucleic acid from said sample;

adding a set of oligonucleotide primer pairs to said isolated sample, wherein said set of oligonucleotide primers comprises at least five oligonucleotide primer pairs, wherein at least one primer pair is capable of specifically amplifying a DNA sequence to produce an amplified product of a virulence factor/toxin gene characteristic for each one of an enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic uenterohemorrhagic pathogenic E. coli strains; and

wherein at least one of set is the primer pair EI-1: 5' TTT CTG GAT GGT ATG GTG AGG 3' (SEQ ID NO: 9) and EI-2: 5' CTT GAA CAT AAG GAA ATA AAC 3' (SEQ ID NO: 10), which specifically hybridizes to the inv-plasmid; and

subjecting said sample and said set of primer pairs to an amplification process; and

detecting the presence of at least one amplified product, wherein the presence of at least one amplified product indicates the presence of at least one pathogenic enterobacteria strain in said sample.

22. (Amended) The method according to claim 21, wherein the set of oligonucleotide primer pairs comprises primer pairs selected from the group consisting of:

U.S.N.: 09/403,690
Attorney Docket No: 20239-0706-US
Client Docket No: BN 25 PCT-US

at least one primer pair that hybridizes to a gene encoding heat labile toxin or a gene encoding heat stable toxin for amplification of a DNA sequence characteristic for enterotoxigenic *E. coli*;

at least one primer pair that hybridizes to a gene encoding heat stable toxin or to a pCDVD432 plasmid for amplification of a DNA sequence characteristic for enteroaggregative *E. coli*;

~~at least one primer pair that hybridizes to a inv plasmid for amplification of a DNA sequence characteristic for enteroinvasive *E. coli*;~~

at least one primer pair that hybridizes to a EAF plasmid, or an eae gene for amplification of a DNA sequence characteristic for enteropathogenic *E. coli*; and

at least one primer pair that hybridizes to genes encoding shiga-like toxin stII or stIII for amplification of a DNA sequence characteristic for enterohemorrhagic *E. coli*.

23. (Amended) The method according to claim 22, wherein the oligonucleotide primer pair that hybridizes to the gene encoding heat labile toxin characteristic for enterotoxigenic *E. coli* is

LT-1: 5' GCG TTA CTA TCC TCT CTA TGT G 3' (SEQ ID NO: 1) and LT-2: 5' AGT TTT CCA TAC TGA TTG CCG C 3' (SEQ ID NO: 2); and

the oligonucleotide primer pair that hybridizes to the gene encoding heat stable toxin characteristic for enterotoxigenic *E. coli* is

ST-1: 5' TCC CTC AGG ATG CTA AAC CAG 3' (SEQ ID NO: 3) and ST-2a: 5' TCG ATT TAT TCA ACA AAG CAA C 3' (SEQ ID NO: 4); and

the oligonucleotide primer pair that hybridizes to the gene encoding heat stable toxin characteristic for enteroaggregative *E. coli* is

EAST1-1: 5' AAC TGC TGG GTA TGT GGC TGG 3' (SEQ ID NO: 5) and EAST1-2: 5' TGC TGA CCT GCC TCT TCC ATG 3' (SEQ ID NO: 6); and

U.S.S.N.: 09/403,690
Attorney Docket No: 20239-0706-US
Client Docket No: BN 25 PCT-US

the oligonucleotide primer pair which hybridizes to the pCVD432 plasmid is

EA-1: 5' CTG GCG AAA GAC TGT ATC ATT G 3' (SEQ ID NO: 7) and EA-2: 5' TAA TGT ATA GAA ATC CGC TGT T 3' (SEQ ID NO: 8); and

~~the oligonucleotide primer pair which hybridizes to the inv plasmid is~~

~~EI-1: 5' TTT CTG GAT GGT ATG GTG AGG 3' (SEQ ID NO: 9) and EI-2: 5' CTT GAA CAT AAG GAA ATA AAC 3' (SEQ ID NO: 10); and~~

the oligonucleotide primer pair which hybridizes to the EAF plasmid is

EP-1: 5' CAG GGT AAA AGA AAG ATG ATA AG 3' (SEQ ID NO: 11) and EP-2: 5' AAT ATG GGG ACC ATG TAT TAT C 3' (SEQ ID NO: 12); and

the oligonucleotide primer pair which hybridizes to the eac gene is

EPEh-1: 5' CCC GCA CCC GGC ACA AGC ATA AG 3' (SEQ ID NO: 13) and EPEh-2: 5' AGT CTC GCC AGT ATT CGC CAC C 3' (SEQ ID NO: 14); and

the oligonucleotide primer pair which hybridizes to the gene encoding shiga-like toxin StII is

StII-1: 5' ATG AAA AAA ACA TTA TTA ATA GC 3' (SEQ ID NO: 15) and StII-2: 5' TCA CYG AGC TAT TCT GAG TCA AGC 3' (SEQ ID NO: 16); and

the oligonucleotide primer pair which hybridizes to the gene encoding shiga-like toxin StIII is

StIII-1: 5' ATG AAG AAG ATR WTT RTD GCR CYT TTA TTY G 3' (SEQ ID NO: 17) and StIII-2: 5' TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC 3' (SEQ ID NO: 18),

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

24. (Reiterated) The method according to claim 21, wherein detecting the presence of at least one amplified product is performed using at least one oligonucleotide probe capable of hybridizing to the amplified product wherein said oligonucleotide probe is labeled at the 5'

U.S.S.N.: 09/403,690
Attorney Docket No: 20239-0706-US
Client Docket No: BN 25 PCT-US

end with a fluorescent reporter dye and at the 3' end with a fluorescent quencher dye and is susceptible to 5'-3' exonuclease degradation by a polymerase, and wherein said amplification process uses a polymerase having 5'-3' exonuclease degradation activity.

25. (Previously Canceled)

26. (Twice Amended) The method according to claim 24 wherein the labeled oligonucleotide probe is selected from the group consisting of:

a labeled oligonucleotide probe specific for the detection of a heat labile toxin gene characteristic for enterotoxigenic E. Coli;

a labeled oligonucleotide probe specific for the detection of a heat stabile toxin gene characteristic for enterotoxigenic E. Coli;

a labeled oligonucleotide probe specific for the detection of a heat stabile toxin gene characteristic for enteroaggregative E. Coli;

a labeled oligonucleotide probe specific for the detection of a pCVD432 plasmid;

~~a labeled oligonucleotide probe specific for the detection of a α -inv plasmid;~~

a labeled oligonucleotide probe specific for the detection of a EAF-plasmid;

a labeled oligonucleotide probe specific for the detection of a eae gene;

a labeled oligonucleotide probe specific for the detection of a shiga-like toxin StII gene; and

a labeled oligonucleotide probe specific for the detection of a shiga-like toxin StII gene.

27. (Reiterated) The method according to claim 26, wherein the labeled oligonucleotide probe for the detection of heat labile toxin gene characteristic for enterotoxigenic E. coli is

5' AGC TCC CCA GTC TAT TAC AGA ACT ATG 3' (SEQ ID NO: 19),

the labeled oligonucleotide probe for the detection of heat stabile toxin gene characteristic for enterotoxigenic E. coli is

5' ACA TAC GTT ACA GAC ATA ATC AGA ATC AG 3' (SEQ ID NO: 20);

U.S.S.N.: 09/403,690
Attorney Docket No: 20239-0706-US
Client Docket No: BN 25 PCT-US

the labeled oligonucleotide probe for the detection of heat stable toxin gene characteristic for enteroaggregative *E. coli* is

5' ATG AAG GGG CGA AGT TCT GGC TCA ATG TGC 3' (SEQ ID NO: 21);

the labeled oligonucleotide probe for the detection of pCVD432 plasmid is

3' CTC TTT TAA CTT ATG ATA TGT AAT GTC TGG 3' (SEQ ID NO: 22);

the labeled oligonucleotide probe for the detection of the inv-plasmid is

5' CAA AAA CAG AAG AAC CTA TGT CTA CCT 3' (SEQ ID NO: 23)

the labeled oligonucleotide probe for the detection of the EAF-plasmid is

5' CTT GGA GTG ATC GAA CGG GAT CCA AAT 3' (SEQ ID NO: 24);

the labeled oligonucleotide probe for the detection of the *eae* gene is

5' TAA ACG GGT ATT ATC AAC AGA AAA ATC C 3' (SEQ ID NO: 25);

the labeled oligonucleotide probe for the detection of shiga-like toxin StII gene is

5' TCG CTG AAT CCC CCT CCA TTA TGA CAG GCA 3' (SEQ ID NO: 26);

and the labeled oligonucleotide probe for the detection of shiga-like toxin StIII gene is

5' CAG GTA CTG GAT TTG ATT GTG ACA GTC ATT 3' (SEQ ID NO: 27).

28. (Reiterated) The method according to claim 24 wherein the fluorescent reporter dye is 6-carboxy-fluorescein, tetrachloro-6-carboxy-fluorescein, or hexachloro-6-carboxy-fluorescein, and the fluorescent quencher dye is 6-carboxytetramethyl-rhodamine.

29. (Reiterated) The method according to claim 21 wherein the amplification process comprises 35 PCR cycles at a $MgCl_2$ concentration of 5.2 mM, an annealing temperature of 55 °C and an extension temperature of 65 °C.

30. (Twice Amended) A set of oligonucleotide primer pairs useful for polymerase chain reaction (PCR) amplification of DNA of pathogenic enterobacteria allowing detection and

U.S.S.N.: 09/403,690
Attorney Docket No: 20239-0706-US
Client Docket No: BN 25 PCT-US

differentiation of pathogenic enterobacteria in a sample wherein following amplification the presence of at least one amplified product indicates the presence of at least one pathogenic enterobacteria strain in said sample, wherein said set comprises at least five primer pairs, wherein each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for one each of the subgroups of the pathogenic E. coli strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains and wherein for amplification of each subgroup at least one of said oligonucleotide primer pair is a primer pair that hybridizes to an inv-plasmid of enteroinvasive E. coli and wherein said primer pair is

EI-1: 5' TTT CTG GAT GGT ATG GTG AGG 3' (SEQ ID NO: 9) and

EI-2: 5' CTT GAA CAT AAG GAA ATA AAC 3' (SEQ ID NO: 10)

included in said set of oligonucleotide primer pairs.

31. (Amended) The set of primer pairs according to claim 30 comprising

a primer pair that hybridizes to a gene encoding heat labile toxin, or to a gene encoding heat stable toxin of enterotoxigenic E. coli;

a primer pair that hybridizes to a gene encoding heat stable toxin or to a pCVD432 plasmid of enteroaggregative E. coli;

~~a primer pair that hybridizes to a inv-plasmid of enteroinvasive E. coli;~~

a primer pair that hybridizes to a EAF plasmid, or a eae gene of enteropathogenic E. coli; and

a primer pair that hybridizes to a gene encoding shiga-like toxin stII or stIII of enterohemorrhagic E. coli.

32. (Amended) The set of primer pairs according to claim 31 wherein

the primer pair which hybridizes to the gene encoding heat labile toxin of enterotoxigenic E. coli is

LT-1: 5' GCG TTA CTA TCC TCT CTA TGT G 3' (SEQ ID NO: 1) and

U.S.S.N.: 09/403,690
Attorney Docket No: 20239-0706-US
Client Docket No: BN 25 PCT-US

LT-2: 5' AGT TTT CCA TAC TGA TTG CCG C 3' (SEQ ID NO: 2);

the primer pair which hybridizes to the gene encoding heat stable toxin of enterotoxigenic E. coli is

ST-1: 5' TCC CTC AGG ATG CTA AAC CAG 3' (SEQ ID NO: 3) and

ST-2a: 5' TCG ATT TAT TCA ACA AAG CAA C 3' (SEQ ID NO: 4);

the primer pair which hybridizes to the gene encoding heat stable toxin of enteroaggregative E. coli is

EAST1-1: 5' AAC TGC TGG GTA TGT GGC TGG 3' (SEQ ID NO: 5) and

EAST1-2: 5' TGC TGA CCT GCC TCT TCC ATG 3' (SEQ ID NO: 6);

the primer pair which hybridizes to the pCVD432 plasmid is

EA-1: 5' CTG GCG AAA GAC TGT ATC ATT G 3' (SEQ ID NO: 7) and

EA-2: 5' TAA TGT ATA GAA ATC CGC TGT T 3' (SEQ ID NO: 8);

the primer pair which hybridizes to the inv plasmid is

~~EL 1: 5' TTT CTG GAT GGT ATG GTG AGG 3' (SEQ ID NO: 9) and~~

~~EL 2: 5' CTT GAA CAT AAG GAA ATA AAC 3' (SEQ ID NO: 10);~~

the primer pair which hybridizes to the EAF plasmid is

EP-1: 5' CAG GGT AAA AGA AAG ATG ATA AG 3' (SEQ ID NO: 11) and

EP-2: 5' AAT ATG GGG ACC ATG TAT TAT C 3' (SEQ ID NO: 12);

the primer pair which hybridizes to the eac gene is

EPeh-1: 5' CCC GGA CCC GGC ACA AGC ATA AG 3' (SEQ ID NO: 13) and

EPeh-2: 5' AGT CTC GCC AGT ATT CGC CAC C 3' (SEQ ID NO: 14);

the primer pair which hybridizes to the shiga-like toxin sltI gene is

U.S.S.N.: 09/403,690
Attorney Docket No: 20239-0706-US
Client Docket No: BN 25 PCT-US

SlitI-1: 5' ATG AAA AAA ACA TTA TTA ATA GC 3' (SEQ ID NO: 15) and

SlitI-2: 5' TCA CYG AGC TAT TCT GAG TCA AGC 3' (SEQ ID NO: 16); and

the primer pair which hybridizes to the shiga-like toxin slitII is

SlitII-1: 5' ATG AAG AAG ATR WTT RTD GCR GYT TTA TTY G 3' (SEQ ID NO: 17)
and

SlitII-2: 5' TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC 3' (SEQ ID NO: 18)

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

33. (Canceled)

34. (Canceled)

35. (Canceled)

36. (Amended) A set of oligonucleotide primer pairs and a set of oligonucleotide primer probes useful for diagnosing an enterobacteria infection in samples derived from a living animal body including a human, by Real time PCR method, wherein said sets of oligonucleotide primer pairs and oligonucleotide primer probes allow detection and differentiation of pathogenic enterobacteria in a sample, wherein said set of oligonucleotide primer pairs comprises at least five primer pairs and at least one primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for each of the subgroups of the pathogenic E. coli strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains and, wherein said set of oligonucleotide probes comprises at least one oligonucleotide probe, each oligonucleotide probe specifically binding a sequence of a virulence factor/toxin genes characteristic of one of the subgroups of pathogenic E. coli strains, and wherein at least one of said set of primer pairs is a set that hybridizes to an inv-plasmid of enteroinvasive E. coli, and wherein said set is

U.S.S.N.: 09/403,690
Attorney Docket No: 20239-0706-US
Client Docket No: BN 25 PCT-US

EL-1: 5' TTT CTG GAT GGT ATG GTG AGG 3' (SEQ ID NO: 9) and

EL-2: 5' CTT GAA CAT AAG GAA ATA AAC 3' (SEQ ID NO: 10); and

said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains by real time PCR.

37. (Reiterated) The method of claim 21, wherein said method is used to diagnose an enterobacteria infection in a sample derived from a living animal body.

38. (Reiterated) The method of claim 37, wherein said sample is derived from a human.

39. (Reiterated) The method of claim 21, wherein said method is used to detect enterobacteria contamination of a consumable.

40. (Reiterated) The method of claim 39, wherein said consumable is selected from the group consisting of meat, milk and vegetable.

U.S.S.N.: 09/403,690
Attorney Docket No: 20239-0706-US
Client Docket No: BN 25 PCT-US